interface with the antigen presenting cell. Recent evidence suggests they are actively signaling, however their role in signal initiation and propagation is still unclear. To mechanically control the agonist MCC-MHC per TCR cluster we use microcorrals of fluid supported lipid bilayer with a confined number of MCC-MHC and correspondingly bound TCR. T cell activation (measured by calcium flux) on these microcorrals is significantly reduced at low MCC-MHC concentrations, compared to cells off them. T cell activation depends not on the overall number of agonist-MHC present, but on their number per microcorral. At least two MCC-MHCs per corral are required to trigger T cell calcium flux. Cells that can cluster even more MCC-MHC per TCR cluster do not exhibit higher signaling. This result provides an extension to the heterodimer hypothesis, by which a direct complex between agonist-MHC-TCR and co-agonist-MHC-TCR is the functional signaling unit. It confirms that two MCC-MHC-TCR are required and suggests that they are sufficient for TCR nanocluster signaling.

3513-Pos Board B560

Spatial Mutation of The T Cell Immunological Synapse with Hybrid Protein and Membrane Patterned Surfaces

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Reorganization of membrane components, such as membrane receptors and adhesion proteins, plays an important role in signal transduction *in vivo*. Patterned hybrid live cell-supported membrane junctions provide spatial controls over the lateral transport of signaling molecules inside the cell. Moreover, immobile protein patterns within hybrid cell junctions can serve as diffusion hindrances with selectivity to their specific ligand on cellular membranes. Here we combine protein and membrane patterning to study molecular assembly during immunological synapse formation. Specifically, we explore the effects of certain fixed obstacles on the overall intracellular actin flow and receptor transport processes. The micro-patterned membranes with the abilities to selectively retain signaling molecules in position enable us to explore dynamical sorting mechanisms in cellular membranes.

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NMR Footprinting Of Activating And Non-activating Monoclonal Antibodies On CD3 Indicate Dynamic Quaternary Structure Changes In The TCR Complex

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The T cell receptor (TCR) mediates antigen recognition and T cell activation via its dimeric αβ, CD3εγ, CD3εδ and CD3ζζ subunits, however, a structural mechanism relating both functions has remained elusive. Here, we determine the NMR footprints on CD3εγ of one non-agonist and two agonist anti-CD3 monoclonal antibodies (mAbs). The data indicate changes of the site-specific binding topology and the TCR quaternary structure upon activation. NMR cross-saturation and chemical shift mapping showed that agonist and non-agonistic mAbs have distinct binding sites on the CD3εγ heterodimer. Agonistic mAbs bind to the membrane distal CD3ε lobe, whereas a non-agonist mAb targets the cleft between CD3ε and CD3γ causing a non-native quaternary structure in TCRβ-CD3εγ module. Subsequent biological experiments confirmed that the difference in cell triggering is not linked to mAb affinity or CD3ε binding stoichiometry per TCR but to the difference in the binding epitope on CD3εγ. More importantly, an Fab that stabilizes an intact TCRβ-CD3εγ module inhibits antigen-dependent activation. These findings indicate that a dynamic but coordinated receptor quaternary structure change in T cell receptor is important for T cell activation, which offer new insights into functional integration within multi-subunit receptors and may guide design of immunosuppressive mAbs devoid of agonist activity.

3515-Pos Board B562

Pharmacological Properties of a Pore Induced by Rising in Intracellular Ca^{2+}

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Abstract

Recent studies on the $P2X_7$ receptor in 2BH4 cells and peritoneal macrophages have demonstrated that a rise in intracellular Ca^{2+} concentration induces a pore opening similar to $P2X_7$ receptor pore. Herein, we have investigated whether the pore activated by rising in intracellular Ca^{2+} concentration is associated to $P2X_7$ receptor. Using patch clamp in cell attached, whole cell configuration and dye uptake, we measured the pore opening in cell types that express the $P2X_7$ receptor (2BH4 cells and peritoneal macrophages), and in cells that do not express this receptor (HEK-293 and IT45-RI cells). In 2BH4 cells, the stimulation with ionomycin (5-10μM) increased intracellular free Ca^{2+} concentration and induced pore formation with conductance of 421 ± 14 pS, $t_{1/2}$ for ethidium bromide (EB) uptake of 118 ± 17 s, and $t_{1/2}$ for Lucifer yellow (LY) of 122 ± 11 s. $P2X_7$ receptor antagonists did not block this effect. Stimulation of HEK-293 and IT45-RI cells resulted in pore formation with properties similar to those found for 2BH4 cells. Connexin hemichannels inhibitors (carbenoxolone and heptanol) also did not inhibit the pore induced effect following rise in intracellular Ca^{2+} concentration. However, 5-(N,N-hexamethylene)- amiloride (HMA), a $P2X_7$ receptor pore blocker, inhibited the induced pore. Moreover, intracellular signalling enzymes, such as calmodulin, phospholipase-C (PLC), mitogen-activated protein kinase (MAPK), and cytoskeleton components were important for the pore formation. Additionally, we confirmed the results obtained for electrophysiology by using the flow cytometry, and we discarded the possibility of cellular death induced by rise of intracellular Ca^{2+}, at the doses used by using lactate dehydrogenase (LDH) release assay. In conclusion, increased mobilization of intracellular Ca^{+2} induces a novel membrane pore pharmacologically different from the $P2X_7$ associated pore and hemichannel pore.

3516-Pos Board B563

Exercise training during diabetes minimizes loss of Rap2 and Rrad

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Diabetes causes failure of multiple organs. However, molecular mechanisms underlying these defects remain incompletely characterized. Recent studies suggest that alterations in expression/function of GTPases maybe involved. This study was designed to determine whether expression of two of these GTPases, Rap2 and Rrad are altered during diabetes and whether exercise training could blunt these changes. Type 1 diabetes was induced in male Sprague-Dawley rats using streptozotocin (STZ). Three weeks after STZ injection, diabetic rats were divided into two groups. One group underwent exercise training (ExT) for four weeks while the other group remained sedentary. After seven weeks of diabetes, steady state levels of Rap2 protein decreased by 50% in heart, 40% reduction in brain, 30% reduction in liver, but there was no detectable change in kidneys. Steady state levels of Rrad protein was also reduced by 60% in heart, 20% in brain and 25% reduction in liver as well as by 50% in kidney during diabetes. Four weeks of ExT initiated three weeks after the onset of diabetes, attenuated the reduction in Rap2 and Rrad expression. Since Rap2 and Rrad are down stream effectors of the guanine nucleotide exchange protein, EPAC which is activated during diabetes, these data suggest that reduction in steady state levels of Rap2 and Rrad may serve to blunt the effect of persistent sympathetic activation. (Supported in part by minority supplement, NIH).

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Cardiac Hypertrophy In Diabetic Mice Is Prevented By Ablation Of The G-protein gα11

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Large clinical studies provided evidence of a lower incidence of a reduced cardiac morbidity in diabetic patients treated with angiotensin type 1 (AT1) receptor blockers. The AT1 receptor is coupled to the Gq class of G-proteins, which stimulate proteinkinase C (PKC) via activation of phospholipase Cβ. To study the role of the Gq protein Gα11 and its signaling through PKC in diabetic heart disease, we induced diabetes in wildtype and Gα11 knockout mice using streptozotocin.

After eight weeks of stable hyperglycemia, cardiac morphology and function were assessed by echocardiography, myocardial expression and translocation of PKC isoforms by immunohistochemistry and immunoblot after tissue fractionation.

Wildtype mice (n=8) but not Gα11 knockout animals (n=8) developed ventricular hypertrophy upon induction of hyperglycemia. PKC isoforms α, βII, δ, ε, ζ and θ were all detected in myocardium of wildtype animals. Compared to normoglycemic control animals, PKC isoforms α and ζ showed increased expression levels in diabetic wildtype mice. In addition, PKC ζ was phosphorylated at Thr410/403 and showed strong translocation to nuclear membranes in cardiomyocytes of diabetic but not of control animals. Hearts from normoglycemic Gα11-knockout mice showed lower expression levels of PKC α and δ compared